the end of a similar 5-ft length of twine. The resulting assembly resembles the letter Y with a 5-ft long vertical stem and two diagonal branches (one 1 ft long and the other 2 ft long), with a folded swab securely attached at the end of each branch. After assembly, place each two-pad drag-swab sampling set into a sterile bag.

(ii) *Procedure for taking drag-swab*—(A) *Floor litter:* The Plan participants should collect two samples as follows: Drag four 3-by-3-inch sterile gauze pads premoistened with double strength skim milk[13] over the floor litter surface for 15 min minimally. Place the gauze pads used to collect the samples in 18-oz whirl-pack bags, two pads per bag with each bag containing 5 ml of double strength skim milk. This will maintain the moistness of the sample during transport. Mark the bags with the type of sample and the house identification.

(B) *Nest-boxes.* The Plan participant should collect one nest-box sample by using two 3-by-3-inch sterile gauze pads premoistened with double strength skim milk. Wipe the two gauze pads used to collect the sample over assorted locations of about 10 percent of the total nesting area. Place the gauze pads used to collect the sample in an 18-oz whirl-pack bag containing 5 ml of double strength skim milk. Mark the bag with the type of sample and the house identification.

(Approved by the Office of Management and Budget under control number 0579–0007)

[38 FR 13709, May 24, 1973. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 57 FR 57342, Dec. 4, 1992; 59 FR 12805, Mar. 18, 1994; 59 FR 67617, Dec. 30, 1994; 61 FR 11524, Mar. 21, 1996; 62 FR 44070, Aug. 19, 1997; 63 FR 3, Jan. 2, 1998; 65 FR 8019, Feb. 17, 2000; 67 FR 8471, Feb. 25, 2002]

§ 147.13 Procedure for bacteriological culturing of eggshells for colon bacilli organisms.

Proper precautions to avoid environmental contamination of the samples during the collection and laboratory process, and proper handling of the samples following collection are essential. Each State Inspector involved in eggshell culture activities must receive instruction in the necessary sanitation procedures, sampling procedures, and sample handling by the authorized laboratory involved. The Official State Agency will maintain a record showing that the required instruction was given to each State Inspector.

(a) *Sample selection.* Forty (40) eggs in the top flats of each of three randomly selected cases of sanitized eggs from each flock will be utilized for each sampling.

(b) *Swab procedure.* A 2.5 centimeter diameter circular area of the large end of each of the eggs will be rubbed with a sterile swab previously moistened with sterile lactose broth, or other suitable liquid media provided by the authorized laboratory. One swab will be used for five eggs, and four swabs will be pooled to each sterile, capped tube provided by the authorized laboratory.

(1) From the tube containing four swabs and lactose broth or other suitable media, 1 ml. will be transferred to 10 ml. lactose in a fermentation tube.

(2) Incubate at 37 °C for 48 hours. The presence of acid, and gas in the amount of 10 percent or more after 24 and 48 hours of incubation, provides a presumptive conclusion of the presence of colon bacilli organisms.

(Approved by the Office of Management and Budget under control number 0579–0007)

[41 FR 14256, Apr. 2, 1976. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 59 FR 12805, Mar. 18, 1994]

§ 147.14 Procedures to determine status and effectiveness of sanitation monitored program.

The following monitoring procedures[14] may be applied at the discretion of the Official State Agency:

[13] Obtain procedure for preparing double strength skim milk from USDA–APHIS ''Recommended Sample Collection Methods for Environmental Samples'' available from the National Poultry Improvement Plan, Veterinary Services, APHIS, USDA, 1498 Klondike Road, Suite 200, Conyers, GA 30094.

[14] Laboratory procedures for monitoring operations proposed here are described in the following two publications: Isolation and Identification of Avian Pathogens, American Association of Avian Pathologists, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania 19348—1692, 1980, and Culture Methods for the Detection of Animal Salmonellosis and Arizonosis,

(a) Monitor effectiveness of sanitation program.

(1) Culture the surface of cased eggs periodically for fecal contaminating organisms as described in § 147.13.

(2) Culture a sample of dead-in-shell eggs periodically from each breeding flock for coliforms. Such eggs should also be cultured for the dependable recovery of *salmonellae*. Culturing for the dependable recovery of *salmonellae* should include the use of:

(i) Preenrichment broths supplemented with 35 mg ferrous sulfate per 1,000 ml preenrichment to block iron-binding, *Salmonella*-inhibiting effects of egg conalbumin; and

(ii) Tetrathionate selective enrichment broths, competitor-controlling plating media (XLT4, BGN, etc.), delayed secondary enrichment procedures, and colony lift assays detailed in paragraph (a)(5) and illustration 2 of § 147.11.

[41 FR 48726, Nov. 5, 1976. Redesignated at 44 FR 61586, Oct. 26, 1979, and amended at 57 FR 57343, Dec. 4, 1992; 59 FR 12805, Mar. 18, 1994; 59 FR 59640, Nov. 18, 1994; 61 FR 11524, 11525, Mar. 21, 1996; 65 FR 8019, Feb. 17, 2000]

§ 147.15 Laboratory procedure recommended for the bacteriological examination of mycoplasma reactors.[15]

(a) Turbinates, trachea, air sacs, sinuses, nasal passages, respiratory exudates, synovial fluid, eggs (including yolk, yolk sacs, membranes and allantoic fluid), should be directly sampled with sterile swabs. Aseptic techniques are very important as some organisms may not be suppressed by the antimicrobial agents used in this procedure. Tissue suspensions from large volumes are sometimes desirable from the sites listed above and occasionally from the oviduct and cloaca. Tissues should be ground or blended completely in 10 times their volume of Mycoplasma Broth Medium (MBM). (See paragraph (f) of this section.) Specimens submitted to referral laboratories in order of preference for recovery of the mycoplasma organisms are: (1) live birds, (2) refrigerated fresh tissues, (3) tissue specimens packed with dry ice.

(b) Inoculate 5–10 ml of MBM with a swab, wire loop or 0.1 ml of the tissue suspension. When evidence of growth is observed (lowered pH or turbidity of broth) transfer each broth culture as needed to maintain the original isolates. Incubate tubes at 37 °C for at least 21 days before discarding as negative. When growth is first observed or if no growth occurs by the *4th or 5th* day of incubation, inoculate broth culture onto a plate of Mycoplasma Agar Medium (MAM). (See paragraph (g) of this section.) Several cultures may be inoculated on one plate by using a wire loop or a cotton swab. Incubate plates 3–5 days at 37 °C in a high humidity chamber. If preferred, 5 percent CO^2 may be added or a candle jar may be used. Tiny circular and translucent colonies with elevated centers are very suggestive of mycoplasma. Indirect lighting and a low power or dissecting microscope are recommended for observation of the colonies as they are rarely more than 0.2–0.3 mm in diameter.

(c) Isolates must be serotyped.

(1) Isolates may be shipped in MBM with ice packs if shipment will be in transit less than 2–3 days. Longer shipments require freezing of the MBM with dry ice, or shipping MAM slants at room temperature. Isolates must have indications of growth before shipment is made.

(2) Isolates may be stored in MBM at −20 °C for 2–3 weeks, or they may be stored at −68 °C for several years.

(d) Alternate method of culture: An overlay enrichment culture for fastidious and sensitive mycoplasma, especially for *M. meleagridis* should be included.

(1) Pour 2–3 ml of MAM into a test tube and tilt the tube until a slant (approximately 45°) is obtained. Other containers are acceptable.

(2) Overlay the slant with sufficient MBM, so that the media (including inoculum) covers the agar slope.

(3) Inoculate the culture as indicated in paragraph (b) of this section.

Iowa State University Press, Ames, Iowa 50010, 1976.

[15] Yoder, H. W., Jr., ''Mycoplasmosis.'' In: Isolation and Identification of Avian Pathogens. (Stephen B. Hitchner, Chairman, Charles H. Domermuth, H. Graham Purchase, James E. Williams.) 1980, pp. 40–42, Creative Printing Company, Inc., Endwell, NY 13760.